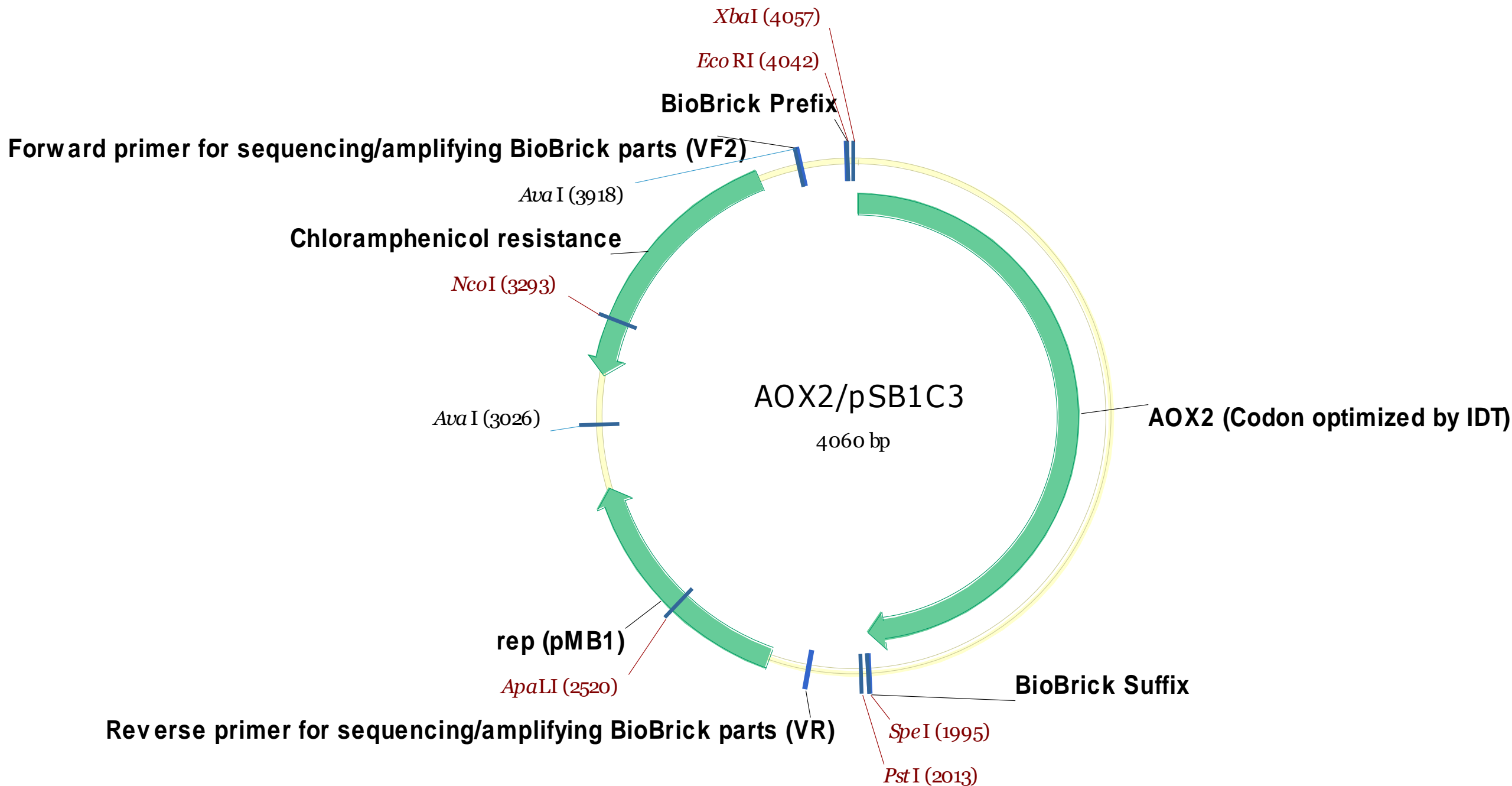


AOX2/pSB1C3



Strategy I → Failed with gBlock

- Vector: pSB1C3 (2070bp) → XbaI + PstI
- Insert: AOX2 (1992 bp) → XbaI + PstI
- PCR with the template of gBlock DNA fragment from IDT

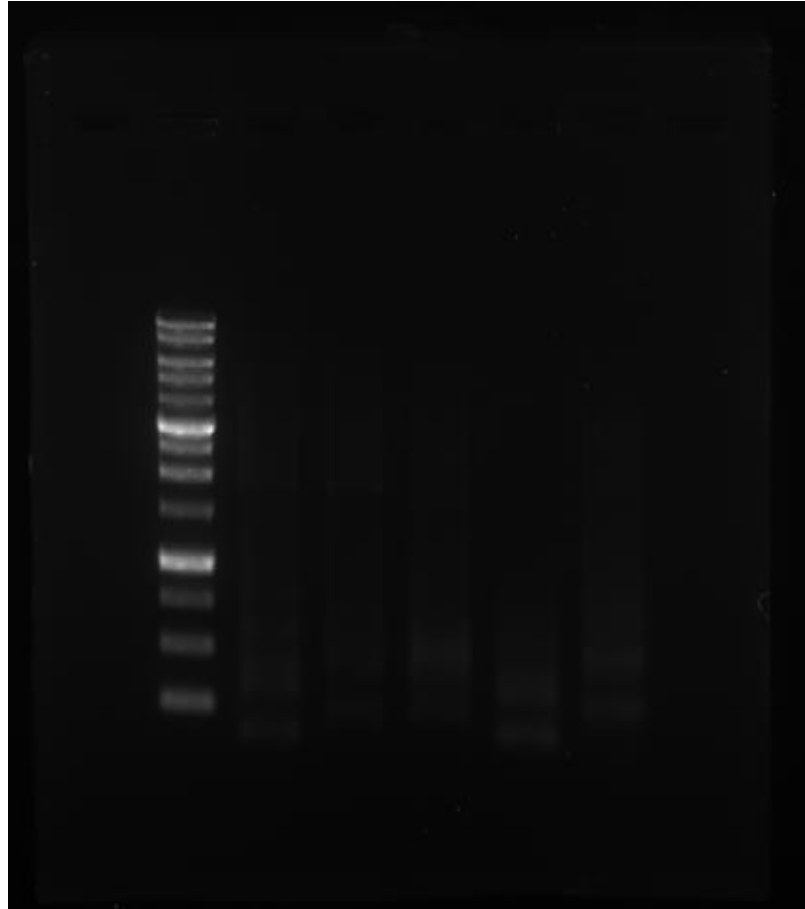
gBlocks® Gene Fragments Entry – AOX2

ATGCCATCCCTGAGGAATTTGACATTTTGGTCTTAGGTGGCGGGTCTCCGGCTCTTGTATTGCAGGACGCTTAG
CGAACCTGGATCACTCGTTAAAGGTTGGATTAATCGAGGCTGGCGAGAACAACCTAAATAACCCGTGGGTTTATCT
GCCAGGGATCTATCCGCGTAATATGAAATTAGATTCAAAAACGGCGTCTTTCTACACTTCTAACCCGTCTCCTCATT
TAAACGGACGCCGCGCTATCGTTCCATGTGCCAATATTTTGGGAGGTGGGAGTTCTATTAATTTTCATGATGTATAC
CCGTGGGTCGGCGTCTGATTACGACGACTTTGAGGCTGAAGGTTGGAAAACAAAGGACCTTTTGCCGCTTATGAA
GAAAACAGAACTTATCAGCGCGCTTGTAACAACCCCGAAATCCACGGTTTTGAGGGCCCAATTAAAGTTTCTTTT
GGGAATTACACTTATCCAGTATGTCAGGATTTCTTACGCGCCACAGAATCCCAGGGTATTCCGTATGTGGATGACT
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TCCTCTTTTCCCATACAGTTCGGAGGCCCGTGCGTACGAGATGGATCTTGAAACGAGTAATGCGTACGGAGGTCCG
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GTTGGTGAGGATCTTGGCTATACCGGCGAGGCATTAGACATGACCGTTCCTCAATTTAAATTGGGAACCTTACGAGA
AGACGGGCTTAGCGCGCTTCTGATACTA

Primer Design

- AOX2-XbaI-F:
 - 5'- GGAGAGTCTAG ATGGCCATCCCTGAGGAATTTG -3'
- AOX2-PstI-R:
 - 5'- AAAAAA CTGCAGCGGCCGCTACTAGTA *TCAGAAGCGCGCTAA* -3'

PCR for AOX1, AOX2, GOX with KOD-plus

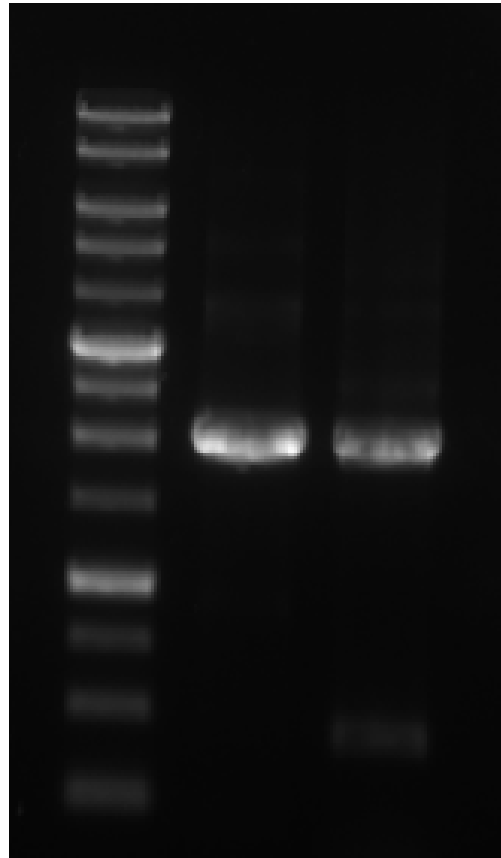


- Lane:
 1. (A1E) AOX1 with AOX1-EcoRI-F / AOX1-HindIII-R
 2. (A2E) AOX2 with AOX2-EcoRI-F / AOX2-HindIII-R
 3. (GE) GOX with GOX-EcoRI-F / GOX-HindIII-R
 4. (A1S) AOX1 with AOX1-XbaI-F / AOX1-PstI-R
 5. (A2S) AOX2 with AOX2-XbaI-F / AOX2-PstI-R
- Cloning: E for pET-29b; S for pSB1C3

Strategy II → Success

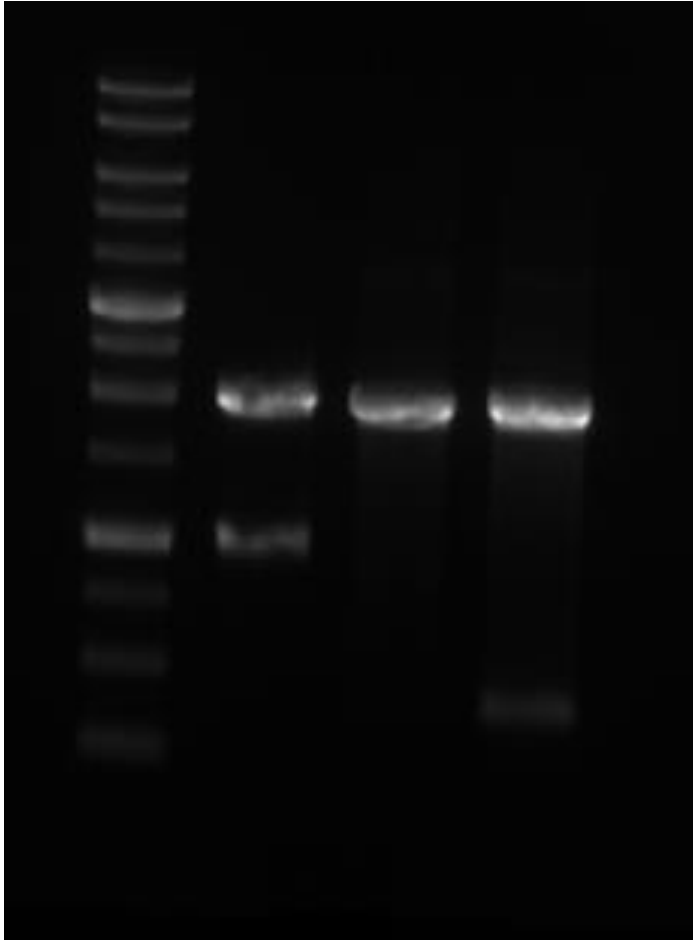
- Vector: pSB1C3 (2070bp) → XbaI + PstI
- Insert: AOX2 (1992 bp) → XbaI + PstI
- PCR with the template of AOX2/pET-29b #3-1
 - Primers: AOX2-XbaI-F & AOX2-PstI-R
 - Annealing T_m: 58 degree C because the reverse primer overlaps template with 12 bp

PCR for AOX1, AOX2 with KOD-plus



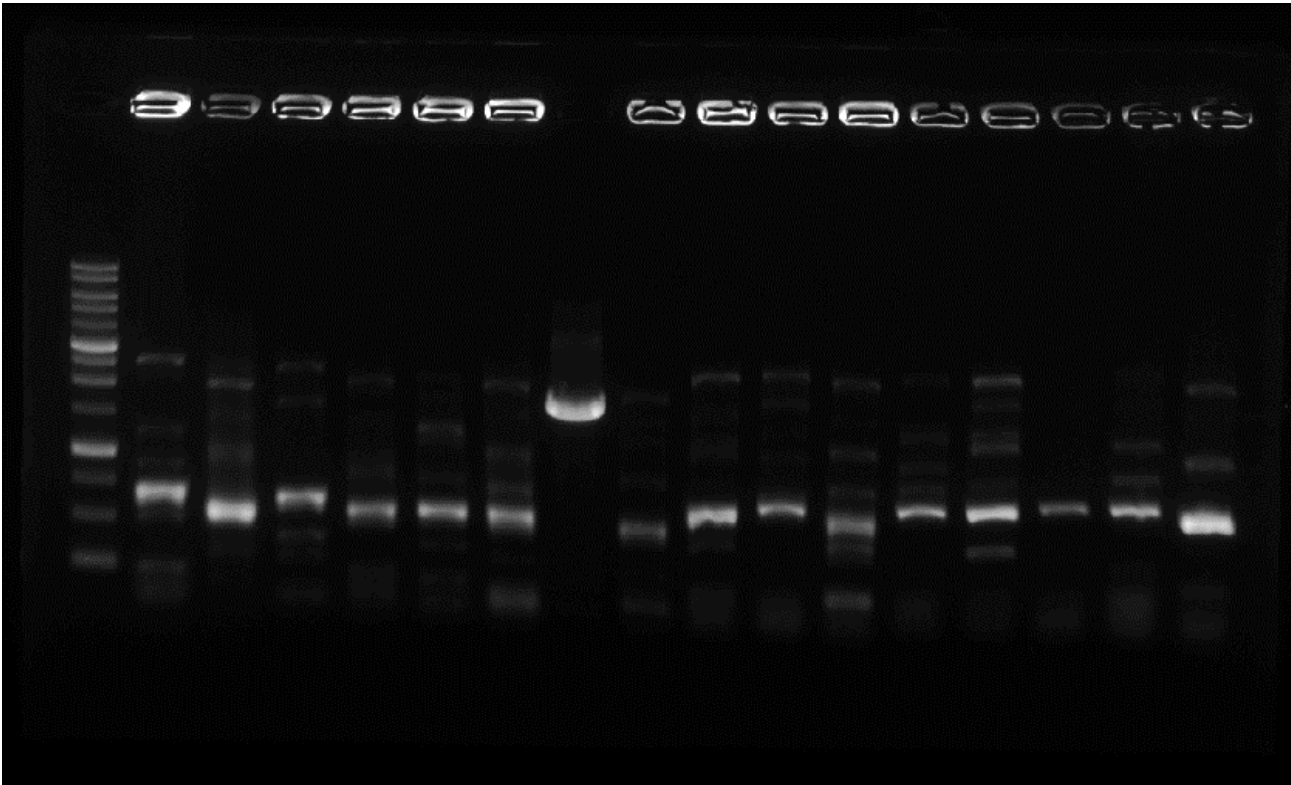
- Date: 2016.08.10
- Lane:
 1. XbaI-AOX1-PstI
 2. XbaI-AOX2-PstI

Ligation: AOX1 + pSB1C3, AOX2 + pSB1C3



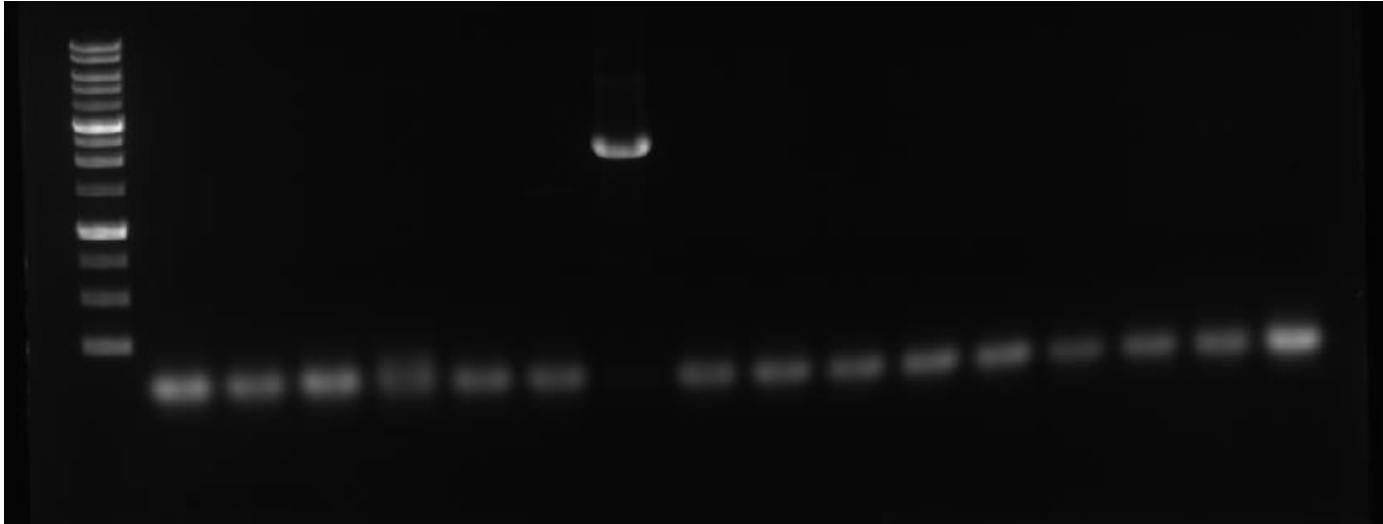
- Date: 2016.08.11
- Lane:
 1. pSB1C3: XbaI + PstI
 2. AOX1: XbaI + PstI
 3. AOX2: XbaI + PstI

Colony PCR



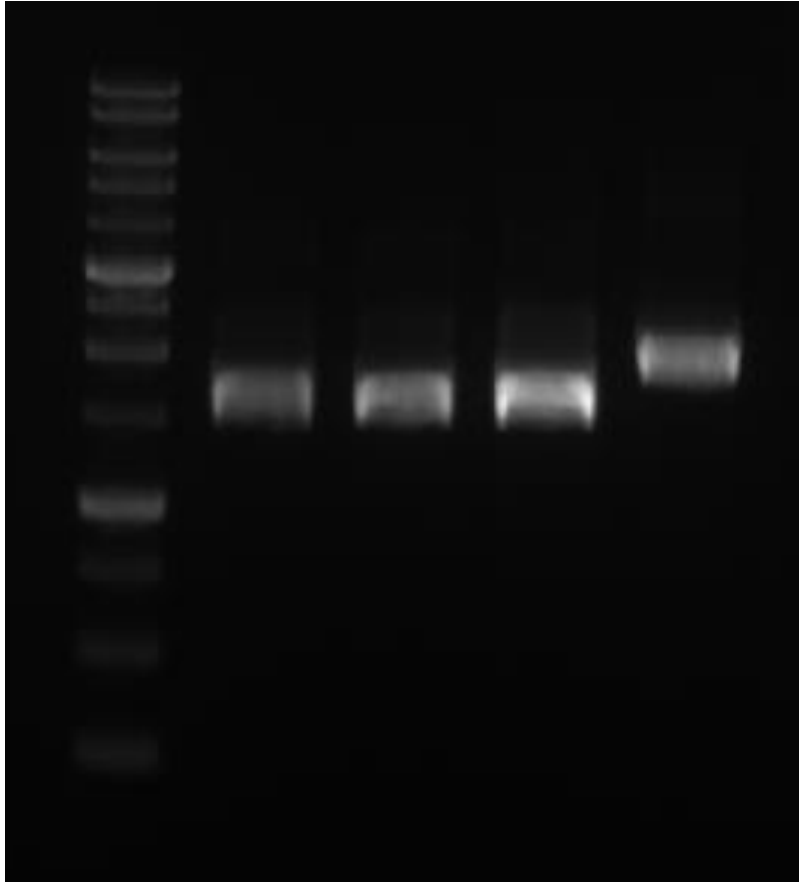
- Date: 2016.08.11
- Clone: AOX2/pSB1C3
- Primer:
 1. AOX2-XbaI-F
 2. AOX2-PstI-R
- Lane: #2-1 ~ #2-16

Colony PCR II



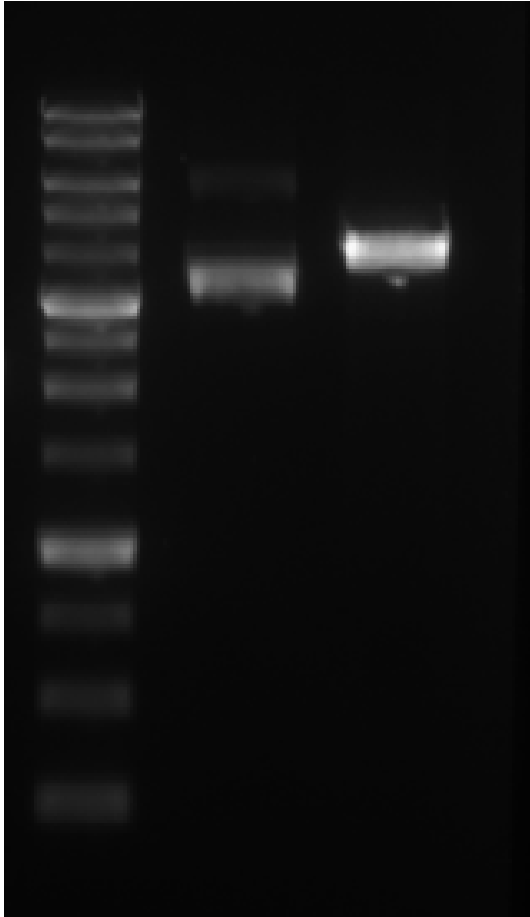
- Date: 2016.08.15
- Clone: AOX2/pSB1C3
- Primer:
 1. AOX2-XbaI-F
 2. AOX2-PstI-R
- Lane: #2-1 ~ #2-16
- #2-7 → Plasmid extraction → RE check

RE check



- Date: 2016.08.16
- Clone: AOX2/pSB1C3
- RE: EcoRI + PstI
- Lane:
 1. #1-2 (AOX1/pSB1C3)
 2. #1-3 (AOX1/pSB1C3)
 3. #1-4 (AOX1/pSB1C3)
 4. #2-7

RE check II



- Date: 2016.08.17
- Clone: AOX2/pSB1C3
- Lane:
 1. #2-7 (uncut)
 2. #2-7 (EcoRI)
- #2-7 → Sequencing

Sequencing

- Date: 2016.08.19
- Sequencing primers: VF2, VR
- Clone: AOX2/pSB1C3 #2-7
- File: “2016.08.19_AOX2_pSB1C3 (#2-7)”
- Result: **correct** → make a stock